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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12Q 1/66, 1/00, C12P 21/06, C07H 21/04, G01N 33/53, 33/567, 33/573, 33/58, 33/60, 33/531, 33/533, 21/00, 21/76, 21/75, 31/00</b>		<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/14271</b> <b>(43) International Publication Date:</b> 16 March 2000 (16.03.00)
<b>(21) International Application Number:</b> PCT/US99/20207 <b>(22) International Filing Date:</b> 2 September 1999 (02.09.99)  <b>(30) Priority Data:</b> 60/099,068 3 September 1998 (03.09.98) US 60/135,835 24 May 1999 (24.05.99) US  <b>(71) Applicant (for all designated States except US):</b> LOMA LINDA UNIVERSITY [US/US]; Loma Linda, CA 92350 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SZALAY, Aladar, A. [US/US]; 7327 Fairwood Lane, Highland, CA 92346 (US). WANG, Yubao [CN/US]; 24931 Prospect Avenue, Loma Linda, CA 92354 (US). WANG-PRUSKI, Gefu [CA/CA]; 114 Hillcrest Avenue, Truro, Nova Scotia B2N 4L3 (CA).  <b>(74) Agents:</b> SHELDON, Jeffrey, G. et al.; Sheldon & Mak, 9th floor, 225 South Lake Avenue, Pasadena, CA 91101 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	
<b>(54) Title:</b> METHOD FOR STUDYING PROTEIN INTERACTIONS <i>IN VIVO</i>			
<b>(57) Abstract</b>  A method for determining whether a first protein interacts with a second protein within a living cell. The method comprises providing the first protein complexed to a donor luciferase and the second protein complexed to an acceptor fluorophore within the cell. The complexed first protein and the complexed second protein are allowed to come into proximity to each other within the cell. Then, any fluorescence from the acceptor fluorophore resulting from luminescence resonance energy transfer from the donor luciferase is detected, where fluorescence from the acceptor fluorophore indicates that the first protein has interacted with the second protein.			
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## PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: JEFFREY G. SHELDON  
SHELDON & MAK  
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9TH FLOOR  
PASADENA, CA 91101

**PCT**

NOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL SEARCH REPORT  
OR THE DECLARATION

(PCT Rule 44.1)

Date of Mailing  
(day/month/year)

**19 NOV 1999**

Applicant's or agent's file reference  
11785-3PCT

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.  
PCT/US99/20207

International filing date  
(day/month/year)  
02 SEPTEMBER 1999

Applicant  
LOMA LINDA UNIVERSITY

1. ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith.
- Filing of amendments and statement under Article 19:**  
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):
- When?** The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.
- Where?** Directly to the International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland  
Facsimile No.: (41-22) 740.14.35
- For more detailed instructions, see the notes on the accompanying sheet.
2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.
3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.
- ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.
4. **Further action(s):** The applicant is reminded of the following:
- Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.
- Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).
- Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

PENSEE T. DO

Telephone No. (703) 308-0196

# TENT COOPERATION TREATY

## PCT

### INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 11785-3PCT	<div style="display: flex; justify-content: space-between;"> <div style="text-align: center;">FOR FURTHER ACTION</div> <div>see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.</div> </div>
International application No. PCT/US99/20207	<div style="display: flex; justify-content: space-between;"> <div>International filing date (<i>day/month/year</i>) 02 SEPTEMBER 1999</div> <div>(Earliest) Priority Date (<i>day/month/year</i>) 03 SEPTEMBER 1998</div> </div>
Applicant LOMA LINDA UNIVERSITY	

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 7 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. ☐ Certain claims were found unsearchable (See Box I).
2. ☐ Unity of invention is lacking (See Box II).
3. ☐ The international application contains disclosure of a nucleotide and/or amino acid sequence listing and the international search was carried out on the basis of the sequence listing
 

☐ filed with the international application.  
☐ furnished by the applicant separately from the international application,  

☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

☐ transcribed by this Authority.
4. With regard to the title, ☒ the text is approved as submitted by the applicant.  
☐ the text has been established by this Authority to read as follows:
5. With regard to the abstract,
 

☒ the text is approved as submitted by the applicant.  
☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the drawings to be published with the abstract is:  
 Figure No. \_\_\_\_\_
 

☐ as suggested by the applicant.  
☐ because the applicant failed to suggest a figure.  
☐ because this figure better characterizes the invention.

☐ None of the figures.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/20207

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
WEST, CAS ONLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, E	US 5,976,796 A (SZALAY et al.) 02 November 1999, col. 1, line 60-col. 3, line 17.	1-32
Y	US 5,491,084 A (CHALFIE et al.) 13 February 1996, col. 1, lines 36-61.	1-32
Y	US 5,418,155 A (CORMIER et al.) 23 May 1995, col. 2, line 59-col. 10, line 9.	1-32
Y, P	US 5,891,646 A (BARAK et al.) 06 April 1999, col. 2, line 6-col. 4, line 47.	1-32
A	US 5,866,348 A (SCHEIRER) 02 February 1999, col. 2, line 15-col. 3, line 27.	1-32

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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  - \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  - \*Z\* document member of the same patent family

Date of the actual completion of the international search  
06 NOVEMBER 1999

Date of mailing of the international search report  
19 NOV 1999

Name and mailing address of the ISA/US  
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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/20207

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,604,364 A (KOSAK) 05 August 1986, col. 3, line 26-col. 8, line 14.	1-32
A	US 4,318,707 A (LITMAN et al.) 09 March 1982, col. 3, line 1-col. 7, line 14.	1-32
A	US 5,683,888 A (CAMPBELL) 04 November 1997, col. 2, line 10-col. 4, line 50.	1-32
A, P	US 5,811,238 A (STEMMER et al.) 22 September 1998, col. 4, line 11-col. 7, line 35.	1-32

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/20207

A. CLASSIFICATION OF SUBJECT MATTER:  
IPC (6):

C12Q 1/66, 1/00; C12P 21/06; C07H 21/04; G01N 33/53, 33/567, 33/573, 33/58, 33/60, 33/531, 33/533, 21/00, 21/76, 21/75, 31/00

A. CLASSIFICATION OF SUBJECT MATTER:  
US CL :

435/4, 7.1, 7.2, 7.21, 7.4, 7.91, 8, 68.1, 69.1, 70.1, 70.3, 127, 231, 232, 326, 331, 366, 375, 968, 972; 436/543, 546, 164, 172, 800, 805, 905; 536/23.5; 424/9.6

B. FIELDS SEARCHED

Minimum documentation searched  
Classification System: U.S.

435/4, 7.1, 7.2, 7.21, 7.4, 7.91, 8, 68.1, 69.1, 70.1, 70.3, 127, 231, 232, 326, 331, 366, 375, 968, 972; 436/543, 546, 164, 172, 800, 805, 905; 536/23.5; 424/9.6